Microvascular and neurodegenerative ocular complications of diabetes mellitus and COVID-19

Ph.D. thesis

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1. Introduction

The severe acute respiratory syndrome caused by coronavirus-2 (SARS-CoV-2) may lead to a spectrum of symptoms ranging from fever and mild cold to severe respiratory failure and death. During the infection, almost 90% of the patients experience neurological symptoms.[1] This neurological involvement may root from the direct involvement of the central or the peripheral nervous system, from the result of inflammatory response caused by the virus, or from increased blood coagulation and endothelial dysfunction.[2] Besides the severe central nervous system problems, cardiovascular events may also occur. SARS-CoV-2 attacks the endothelial cells, therefore micro- and macrovascular complications may develop both on the arterial and the venous side.[3] It is known that coronavirus disease 2019 (COVID-19) may also have ocular complications. The ocular surface is highly exposed to a range of environmental factors and infectious diseases. The tear film might be of importance regarding virus replication in case of SARS-CoV-2 infection. Besides epithelial cells, stromal keratocytes and endothelial cells, a diverse group of antigen presenting cells (APCs) can be identified in the healthy cornea including dendritic cells (DC) and macrophages. DCs are a heterogeneous population of bone marrow-derived APCs and have a pivotal role both in innate and adaptive immunity. After detecting the antigen, the dendritic cells go through a maturation process and they migrate from the meeting point with the antigen to the lymphoid organs, where they stimulate T lymphocytes. Corneal DCs reside on the level of the basal epithelium and the subbasal nerve plexus. In cases of infectious and inflammatory diseases or trauma, large numbers of DCs are recruited from the peripheral cornea and migrate towards the central cornea. Experimental models have confirmed the maturation and phenotypic changes of resident epithelial DCs upon inflammation.[4]

There are two main types of diabetes mellitus (DM): type 1 (T1DM) and type 2 (T2DM) diabetes. T2DM is the more frequent form affecting around 90-95% of the patients. According to the database of the International Diabetes Federation, in 2021 there were 537 million adults with diabetes in the world, and 67 million in Europe. Diabetes might result in macrovascular and microvascular complications. The well-known triad of microvascular complications consists of nephropathy, neuropathy, and retinopathy. The endothelial dysfunction caused by increased blood glucose levels plays a key role in the development of both macro- and microvascular complications. Diabetes may damage every tissue of the eye. Small fiber polyneuropathy and retinopathy are long-term microvascular complications of diabetes

mellitus. Diabetic retinopathy (DR) is the most frequent ocular complication of DM and is a leading cause of blindness in our society. Although DR is considered to be microvascular retinal damage in general, several studies allow for the conclusion that it is indeed a neurovascular disease of the retina.[5]

The presence of DR is associated with increased risk for systemic vascular complications as well. The alterations detected during ophthalmic examination may not only reflect the condition of the eyeglobe, but the whole body too. Retinal microvascular damage shows close correlation with the severity of diabetic polyneuropathy. The trigger factor behind diabetic corneal neuropathy is the trigeminal nerve involvement caused by persistent hyperglycemia resulting in the damage of corneal nerves.

Modern ophthalmic examination procedures are able to qualify and quantify the retinal capillary network, the choriocapillaris, the optic nerve head, and corneal peripheral nerves. Previous research has shown that in vivo confocal microscopy (IVCM) is capable of revealing early corneal subbasal nerve fiber morphology changes – even in the absence of other ophthalmic or systemic manifestations in the fundus and long before the appearance of neuropathy, retinopathy, or microalbuminuria. IVCM is able to provide qualitative and quantitative analysis of peripheral nerves and inflammatory cells of the cornea in health and disease. Optical coherence tomography angiography (OCTA) provides a direct, non-invasive visualization and quantification of the retinal microvasculature and blood flow without administering a contrast material. During imaging process, the OCTA compares the movement of red blood cells in blood vessels to surrounding static structures. In case of diabetes, capillary obstruction and non-perfusion are some of the early events leading to ischemia and tissue damage. The microvascular and neurodegenerative complications of the multisystemic disease caused by COVID-19 pandemic are increasingly widely studied. The disease is known to have an ophthalmologic manifestation or sub phenomenon. Regarding their pathophysiology, diabetes and COVID-19 disease may lead to similar complications in several aspects.

2. Aims

The purpose of this research was to examine retinal and corneal neurodegenerative and retinal microvascular changes with non-invasive clinical methods in patients who had mild COVID-19 disease, and to analyze the correlation between these changes. The study also aimed for examining the cellular and ultrastructural alterations of the cornea, and for quantifying the neuroinflammatory processes following the COVID-19 disease.

A further objective of the research was analyzing the retinal and corneal neurodegenerative and the retinal microvascular alterations of patients with type 1 and type 2 diabetes with non-invasive clinical methods. We are planning to develop biomarkers that signal the progressive course and allow for the prevention of irreversible retinal and corneal complications. The detection of the ophthalmic manifestation is of therapeutic importance in order to prevent subsequent morbidity. The research also intends to shed light on whether retinal microvascular or corneal neurodegenerative alterations emerge earlier in the case of patients with diabetes.

Main hypotheses of the research:

1. Various degrees of nerve fiber morphology alterations can be detected using in vivo confocal microscopy, while with OCT angiography, probable microvascular alterations can be observed in patients with documented history of SARS-CoV-2 infection.

2. Severity of cellular and subbasal nerve alterations detected by in vivo confocal microscopy is related to the grade of diabetes mellitus and other clinical complications.

3. The retinal capillary plexus anomalies detected with OCT angiography are already observable at an early stage of diabetes mellitus, and the severity of the alterations is in correlation with the stage of the underlying condition.

4. At the same time with the alterations detected by confocal microscopy, retinal microvascular changes can be seen before any clinical symptoms or signs which may worsen with increasing severity of retinopathy.

3. Patients and methods

3.1. Patients

3.1.1. Analysis of microvascular and neurodegenerative ocular complications of mild COVID-19

In the study, 63 subjects were prospectively enrolled: 35 patients after polymerase chain reaction (PCR)-proven SARS-CoV-2 infection with mild disease presentation not requiring hospitalization, and 28 healthy age-matched controls. The participants of the research were not vaccinated against the COVID-19 disease. Control subjects had no past or current history of any systemic or ocular disease, and no participant in either group had a history of contact lens wear or intraocular surgery.

3.1.2. Analysis of corneal cellular and neuroinflammatory changes after SARS-CoV-2 infection

71 subjects were enrolled in this study: 30 patients after PCR-proven SARS-CoV-2 infection and 41 healthy age-matched controls. The participants of the research were not vaccinated against the COVID-19 disease. Control subjects had no history of systemic diseases. Patients after SARS-CoV-2 infection were excluded from the analysis, if they had any pre-existing general disorder including metabolic and cardiovascular diseases. No subject in either group had a past or current history of ocular disease, contact lens wear or intraocular surgery.

3.1.3. Analysis of microvascular and neurodegenerative complications of diabetes mellitus

This cross-sectional study comprised thirty-five eyes of 35 healthy volunteers and fifty-two eyes of 52 patients with T1DM (10 patients) and T2DM (42 patients). Subjects had a negative history of ocular surgery, of trauma, and of present or prior ophthalmic disease other than refractive errors (less than ± 3.0 D spherical and cylindrical power). In the diabetic group mild non-proliferative diabetic retinopathy was permitted, but diabetic macular oedema was an exclusion criterium. Control subjects had no past or current history of any systemic disease. The International Clinical Diabetic Retinopathy Disease Severity Scale was used to classify the stage of diabetic retinopathy.

3.2. Methods

In all study groups, the research protocol included visual acuity measurement, slit lamp examination, indirect ophthalmoscopy, intraocular pressure measurement, anterior and posterior segment imaging with anterior (Anterion, Heidelberg Engineering, Heidelberg, Germany) and posterior segment optical coherence tomography (OCT), OCT angiography (DRI OCT Triton Plus, Topcon, Tokyo, Japan), in vivo confocal microscopy (Heidelberg Retina Tomograph II Rostock Cornea Module, Heidelberg Engineering GmbH, Heidelberg, Germany) and color fundus photography. The examinations were performed on both eyes of the participants and the data of a randomly chosen eye were analyzed.

For the segmentation and quantitative analysis of retinal capillary plexuses, the built-in software of the OCTA (IMAGEnet 6 Version 1.26.16898, Topcon) was used. The foveal avascular zone (FAZ) was manually outlined in the OCTA image of the superficial capillary plexus (SCP). The retinal nerve fiber layer (RNFL) thickness was determined in a 3.4 mm radius circle centered at the optic nerve head on the 3D Disc OCT images with the help of the in-built software of the device. On the HD Raster OCT images centered at the macula, the ganglion cell complex thickness (GCL), the central retina thickness, and the central choroidal thickness were evaluated with the help of the automated segmentation OCT map.

Of each examined eye, three good quality IVCM images of the subbasal nerve plexus were selected and analyzed by using the ACCMetrics software V3 (University of Manchester, Manchester, UK) to quantify the corneal nerves. Corneal subbasal nerve fiber density, nerve branch density, nerve fiber length, nerve fiber total branch density, nerve fiber area, and nerve fiber width were calculated. In the diabetic group, the fractal dimension was also analyzed. The instrument-based software was utilized to analyze the density of the basal epithelial cells, the endothelial cells, the anterior and posterior stromal keratocytes, and the dendritic cells. Dendritic cells were counted manually in the central corneal epithelium. To measure the DC area on IVCM images the ImageJ software (http://imagej.nih.gov/ij/; National Institutes of Health, Bethesda, MD, USA) was applied.

4. Results

4.1. Analysis of microvascular and neurodegenerative ocular complications of mild COVID-19 disease

The mean time between the first positive PCR test and the ophthalmic examination was 13.46 ± 6.12 weeks. Significantly lower corneal subbasal nerve fiber density ($P = 0.0009$), nerve branch density ($P = 0.0004$), nerve fiber length ($P < 0.0001$), nerve fiber total branch density ($P = 0.002$) and nerve fiber area ($P = 0.0001$) values were observed in patients after COVID-19 disease compared to healthy controls. There was no significant difference in nerve fiber width between the two groups ($P = 0.421$). Central choroidal thickness was higher in the control group, but no statistically significant difference was found between the two groups ($P = 0.101$). The vascular density (VD) of the temporal SCP was significantly lower in the post-COVID group, but no other SCP and deep capillary plexus (DCP) vessel density parameter differed significantly between the two groups. There was no significant difference in FAZ area between the two groups ($P = 0.582$). RNFL thickness was higher in the control group, but none of the RNFL-GCL complex parameters showed significant difference between healthy and post-COVID subjects.

4.2. Analysis of corneal cellular and neuroinflammatory changes after SARS-CoV-2 infection

The mean time between the PCR-proven diagnosis and the ophthalmic examination was 13.93 ± 6.13 weeks. There was no significant difference in the epithelial ($P = 0.091$), endothelial ($P = 0.519$) and anterior stromal keratocyte cell density ($P = 0.693$) between the two groups. The posterior stromal keratocyte density was significantly lower in patients after SARS-CoV-2 infection ($P = 0.0006$). Dendritic cell density in the central cornea was significantly higher in patients after SARS-CoV-2 infection ($P = 0.0004$), and there was a significant difference in dendritic cell area between the two groups ($P < 0.0001$) as well. Significantly altered subbasal nerve fiber morphology was detected in patients after SARS-CoV-2 infection compared to healthy volunteers for all parameters except nerve fiber width which did not differ significantly between the two groups ($P = 0.116$). Hyperreflective, round inflammatory cells without dendrites were also identified in patients after SARS-CoV-2 infection. 6 patients after SARS-CoV-2 demonstrated subbasal and stromal microneuromas. In the post-COVID group, other alterations observed on IVCM included increased subbasal nerve tortuosity, discontinuity of the nerve fibers, and apparent beading of the stromal nerves.

4.3. Analysis of microvascular and neurodegenerative complications of diabetes mellitus

In the diabetes group, the mean disease duration was 11.17 ± 11.73 years. T1DM was diagnosed in 10 patients and T2DM was found in 42 patients. The mean HbA1c was 7.28% ± 1.33%. In the diabetes group, 44 patients had no apparent DR and 8 patients had mild non-proliferative DR (few microaneurysms). When examining the anterior segment, no alteration was found. All corneal subbasal nerve fiber parameters were decreased in patients with diabetes mellitus compared to healthy subjects, and the difference was significant for each result except for nerve fiber width ($P = 0.586$). No significant difference was observed in central retinal thickness between the control and diabetes group ($P = 0.089$). Central choroidal thickness was lower in patients with diabetes mellitus ($P = 0.016$). The vessel density in the SCP was significantly decreased in the superior ($P <0.0001$), the temporal ($P = 0.001$), and the nasal quadrant ($P = 0.003$) in the diabetes group. In the DCP, only superior VD ($P = 0.036$) decreased significantly in the diabetes group. There was an enlargement in the FAZ area measured in the SCP in the diabetes group, but the difference was only borderline compared to the healthy group ($P = 0.051$). GCL showed a significantly lower value in patients with diabetes mellitus ($P <0.0001$). Significant inverse correlation was observed between disease duration and the vessel density of the SCP in the superior ($r = -0.539$, $P <0.0001$), the temporal ($r = -0.557$, $P <0.0001$), the inferior ($r = -0.433$, $P = 0.005$), and the nasal quadrant ($r = -0.372$, $P = 0.015$), and between disease duration and the vessel density of the DCP in the inferior ($r = -0.369$, $P = 0.019$) and the nasal quadrant ($r = -0.458$, $P = 0.003$).

5. Discussion

5.1. Analysis of microvascular and neurodegenerative ocular complications of mild COVID-19

In the post-COVID group, we identified a decreased number of primary branch points on the main nerve fibers (nerve branch density) and a lower total number of branch points confirming the distal loss of nerve branches (nerve fiber total branch density) with normal nerve fiber width. There was a significant reduction in the number of nerve fibers (nerve fiber density) reflecting the more proximal nerves after COVID-19. Consequently, a significantly decreased total nerve fiber area was observed in the post-COVID group. Based on prior literature, nerve fiber density, nerve fiber area, nerve fiber length, and nerve fiber branch density are of high importance in the diagnosis of neuropathy, while nerve fiber width signals the degree of severity.[6] In the post-COVID group, the nerve fiber damage detected besides normal nerve fiber width may indicate mild small fiber neuropathy. Retinal neurodegeneration was also examined. The analysis of the GCL-RNFL complex indicated no quantifiable neuronal loss. We also studied microvascular changes in the retinal capillary network and choriocapillaris after SARS-CoV-2 infection. Following the COVID-19 disease, in general, we did not observe significant difference in vascular density either in the SCP or in the DCP when compared to the healthy group. Only the VD of the temporal SCP decreased significantly in patients after COVID-19. According to the literature, in patients with diabetes, the VD in the temporal perifoveal region is the most sensitive for early detection of retinopathy which is explained by the anatomic arrangement of the retinal vasculature.[7] Besides an insignificant enlargement of the FAZ, a meta-analysis of 12 examinations found significantly decreased vascular density in the DCP after COVID-19 infection.[8] It is known that severe COVID-19 infection may result in microvascular and thromboembolic complications, and therefore the follow-up of possible retinal microvascular alterations requires increased attention. In our study, different degrees of corneal subbasal nerve fiber morphology alterations could be detected with in vivo confocal microscopy in patients who had PCR-proven mild or asymptomatic SARS-CoV-2 infection. No relevant microvascular changes could be observed with OCT angiography, and structural GCL-RNFL complex parameters did not show any signs of optic neuropathy in post-COVID patients.

Our results suggest that peripheral neurodegenerative changes may occur even after mild or asymptomatic SARS-CoV-2 infection. In vivo confocal microscopy seems to be an important tool in monitoring peripheral neuropathy in patients after COVID-19.

5.2. Analysis of corneal cellular and neuroinflammatory changes after SARS-CoV-2 infection

In our study, a significantly higher DC density was found in patients after mild COVID-19 when compared to healthy volunteers. In patients after SARS-CoV-2, we observed three times as high DC density as in healthy participants. Previous studies report that branched DCs are more likely immunologically mature, while round cells without dendrites may be immunologically immature DCs.[9] We were able to identify both mature DCs and immature round cells without dendrites after SARS-CoV-2 infection. Besides evaluating central corneal DC density and morphology, corneal subbasal nerve fiber degeneration was also studied after SARS-CoV-2 infection. IVCM demonstrated clinically and statistically significant nerve fiber loss and morphology changes after COVID-19. In patients after SARS-CoV-2 infection, we identified microneuromas in the subbasal nerve plexus and stromal nerves. Microneuromas could be the consequences of nerve damage and signs of nerve regeneration.[10] Corneal microneuromas after COVID-19 could be attributed to inflammatory and immune-mediated processes that could cause nerve damage.[11] Further nerve fiber morphologic alterations after SARS-CoV-2 infection include increased tortuosity, discontinuity, and beading. In patients with inflammatory ocular surface diseases, increased tortuosity of subbasal nerve fibers is thought to result from regeneration processes.[12] Nerve beading has been reported in relation to neuropathic corneal pain but also in healthy individuals; it is thought to reflect higher metabolic activity.[13,14] The basal epithelial, anterior stromal keratocyte, and endothelial cell density of the cornea did not show any significant changes after SARS-CoV-2 infection. We observed a reduction in posterior keratocyte density after COVID-19. Lower posterior keratocyte density was shown to be associated with corneal nerve loss in adults with diabetes mellitus.[15] Peripheral small fiber damage could be detected besides the presence of inflammatory dendritic cells after mild COVID-19.

The most conspicuous finding of this study was that the proportion of mature dendritic cells in the central cornea more than tripled after SARS-CoV-2 infection compared to healthy volunteers. A significant loss and altered morphology of subbasal nerve fibers were also observed after COVID-19 disease indicating nerve damage and regeneration. Corneal cellular and ultrastructural changes imply neuroinflammatory consequences of COVID-19 disease in the cornea.

5.3. Analysis of microvascular and neurodegenerative complications of diabetes mellitus

Altered subbasal nerve fiber morphology was found in DM patients even in the lack of DR. In a previous study involving young T1DM patients, we observed significantly lower nerve fiber total branch density confirming an early, more distal loss of nerve branches, consistent with the loss of thinner, more distal branches. Total nerve fiber area was comparable between control subjects and patients with diabetes, presumably due to the early relative preservation of main nerve fibers. In the present study, nerve fiber width did not differ significantly between diabetic and healthy subjects supporting our previous explanation on early, more pronounced loss of distal nerve branches. In our study, the retinal vessel density in the SCP was significantly decreased in the superior, temporal, and nasal quadrant in patients with diabetes. In the DCP, only the superior vascular density decreased significantly in patients with diabetes mellitus. In line with other authors, we detected early retinal microvascular changes with OCTA before the occurrence of ophthalmoscopic alterations. Prior research identified the parafoveal VD of the SCP as a predicting biomarker for visual impairment in DM.[16] Significant FAZ increase in the SCP was also observed in our patients with diabetes. The ganglion cell complex thickness was significantly decreased in our DM group. Accordingly, authors describe correlations between ganglion cell body loss, retinal vasculature changes, and the severity of DR.[17] Previous papers highlight the association between neuronal degeneration and microvascular changes in diabetes mellitus. GCL and RNFL thickness change in early DM has been identified as neuroretinal degeneration that may predict microvascular alterations.[18] In a recent study, a clear interaction was observed between age or age at diagnosis, diabetes duration, and the risk of microvascular events; the greatest risks was found in the youngest ages with the longest disease duration.[19] Our correlation analysis confirms these results: in the diabetic group, the capillary density in all 4 quadrants of the SCP, and the inferior and nasal quadrants of the DCP decreased proportionally with the disease duration.

6. Summary of novel findings

As the result of our research, we were the first to report data on simultaneous examinations performed by OCT angiography and in vivo confocal microscopy aiming for concurrently detecting retinal microvascular damage and corneal neurodegeneration.

1. In our study, we explored the microvascular and neurodegenerative alterations in patients after mild or asymptomatic SARS-CoV-2 infection not requiring hospitalization. In patients after COVID-19 disease, significant decrease and altered morphology was detected in corneal subbasal nerve fiber parameters without retinal neurodegenerative and microvascular alterations. As the result of our complex analysis, we were the first to publish corneal and retinal neurodegeneration, retinal microvascular alterations, and their correlation after SARS-CoV-2 infection in the literature.

2. After SARS-CoV-2 infection, signs of nerve damage and regeneration were identified in the corneal subbasal nerve plexus. We detected microneuromas in the subbasal nerve plexus and in the level of stromal nerves as well. Significantly higher corneal dendritic cell density and area were observed in patients after mild COVID-19 compared to healthy controls. We were the first to publish the corneal cellular and ultrastructural alterations after SARS-CoV-2 infection, which alterations may demonstrate the neuroinflammatory consequences of the disease in the cornea even in the lack of any other ophthalmic changes.

3. In our study, we observed that a significant change in corneal subbasal nerve fiber morphology and retinal capillary density occurred in diabetes mellitus – even before the appearance of ophthalmoscopically visible fundus alterations. Our results implicate a more pronounced and earlier damage to the corneal nerve fibers compared to the retinal microvasculature in patients with diabetes mellitus. We were the first to report the concurrent damage of the corneal subbasal nerve plexus and the retinal capillary network in the same patient population with diabetes mellitus.

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